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Quantitative and qualitative aspects of peroxidases extracted from cladodes of *Opuntia ficus indica*

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Abstract

Peroxidases (EC 1.11.1.7) were extracted from fresh cladodes harvested from nine ecotypes of the cactus species *Opuntia ficus indica* Mill. growing as a collection, in Marrakech (South Morocco). Two enzyme fractions were obtained by a progressive solubility method leading to soluble peroxidases (S) and ionically wall-bound peroxidases (I). The preferred substrate for cladode peroxidases was determined to be *o*-dianisidine over 4-chloro-1-naphtol and guaiacol. Contrarily to roots, no guaiacol-based activity was found in cladodes. The late ecotypes 1'Haddaouia' and 'Moussa' showed a relatively high peroxidase ratio S/I (units g^{-1} fresh weight). When subjected to electrophoresis on polyacrylamide gels, S and I peroxidase fractions each exhibited two enzyme forms based on their electric charges in basic and acidic gel media. Acidic peroxidase forms, well separated on basic gels, showed two principal migration zones with great differences in their enzyme activities depending on the fraction types S and I. Acidic ionically wall-bound peroxidases exhibited fast, highly active isoforms with R_f values 0.42–0.58. Basic forms, represented essentially in fractions S and resolved on acidic gels, were typified by slow and fast isoforms with a double banded pattern in most ecotypes. *Opuntia* ecotypes collected from localities surrounding Marrakech exhibited fast basic soluble isoforms, while distant ecotypes from Marrakech, including late ones, were typified by a fast acidic ionically bound isoperoxidase of R_f 0.58. Possible roles of *O. f. indica* peroxidases in growth and their evaluation as markers in this cactus species are discussed in this study.

Keywords: Cactus; *Opuntia ficus indica*; Peroxidase; Electrophoresis; Ecotypes

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1. Introduction

Opuntia ficus indica (L.) Miller is a cactus species widely utilized in semi-arid zones as a fruit and forage crop. It is one of the few crops that can be cultivated in areas which offer little growth possibility for common fruits and vegetables. Nutritive value of its fruits (prickly pear) and cladodes continues to be studied in attempt to better use this species (Saenz, 2000; Saenz et al., 1998). Research studies related to the histological and physiological aspects of the growth and adaptation of *O. f. indica* to drought were carried out by several authors (Linton and Nobel, 1999; Wang et al., 1998; North and Nobel, 1997; Wang and Nobel, 1996).

In Tiznit (south Morocco), *O. f. indica* species is represented essentially by two ecotypes called 'Aissa' and 'Moussa'. They could be distinguished by the flowering time and the period of fruit maturation which is respectively June to August and September to December (Dehbi and Radouane, 2000). In addition, the presence and absence of spines on cladodes is a frequently used character in the ecotype identification. However, the expression of such characters could be more or less influenced by the environmental conditions. At actual time there is no marker-assisted breeding program for *O. f. indica*.

Peroxidases are oxidoreductases which use hydrogen peroxide or substituted peroxides for the oxidation of a large number of substrates. They have been widely used as markers in the plant kingdom, due to their low cost and their high polymorphism (Obinger et al., 1996). For many plant species such as date palm (*Phoenix dactylifera* L.) these enzymes were represented essentially by two important active fractions, which were soluble and ionically wall-bound enzymes (Baaziz et al., 1994; Majourhat et al., 2002). Peroxidase chages with relation to flowering and fruit growth and maturation were studied in many plants, such as satsuma mandarin (Monerri and Guardiola, 2001), peach (Abeles and Biles, 1991), grapes (Ros Barcelo et al., 1994), Chile pepper (Biles et al., 1997) and tomato (Anderews et al., 2002). Studies on peroxidases of cactaceae are still not well developed. Padiglia et al. (1995) have purified and characterized an *O. f. indica* peroxidase of molecular weight 58,000 Da obtained from mature fruits. Data regarding cladode peroxidases are absent.

In this work, quantitative and qualitative aspects of *O. f. indica* peroxidases extracted from cladodes were studied respectively using spectrophotometry and electrophoresis techniques. The objective of this work is to evaluate these oxidoreductases in this plant part and to show possible quantitative and qualitative differences and similarities of these enzymes obtained from many *O. f. indica* ecotypes, which differ for some morphological characters and fruit maturation period.

2. Material and methods

2.1. Plant material

Cladodes of prickly pear (*O. f. indica* Mill.) were harvested from nine ecotypes growing in Marrakech (31° latitude North, 4° longitude West, precipitations of 240 mm per year) and previously collected from six different localities. Ecotype culture in Marrakech locality allows plant growth in a homogenous soil and the same environmental conditions.